

## **PDF hosted at the Radboud Repository of the Radboud University Nijmegen**

This full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/15918>

Please be advised that this information was generated on 2014-11-12 and may be subject to change.



## Physiological Ecology of Riverside Species: Adaptive Responses of Plants to Submergence

C. W. P. M. BLOM, L. A. C. J. VOESENEK, M. BANGA, W. M. H. G. ENGELAAR,  
J. H. G. M. RIJNDERS, H. M. VAN DE STEEG and E. J. W. VISSER

Department of Ecology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

Accepted: 10 February 1994

In river floodplains, variation in flooding conditions results in successional stages in colonization ranging from annual pioneers to long-lived perennials. Reactions to submergence of species from the mid-successional zone are compared with adaptive responses of species from other zones. Presence and abundance are related to elevation and can be explained by characteristics of biomass production, and recovery in response to various submergence intensities.

*Rumex* species, from early to late successional stages, serve as models to elucidate, in more detail, mechanisms of adaptation. Flooding-resistant species develop large numbers of adventitious roots upon submergence and exposure to low oxygen conditions. Due to internal oxygen transport through aerenchyma, soil around these roots is re-oxidized, which stimulates bacterial nitrification. Ethylene and auxin promote adventitious rooting. Increased petiole elongation is also an adaptive feature of submergence-resistant *Rumex* species. Differences between species in submergence-induced growth are not only controlled by variation in endogenous levels of ethylene but also by different sensitivities to this hormone. Auxin does not affect *Rumex* petiole elongation, but a clear positive effect of gibberellin is demonstrated. Apparently, submergence induces a higher sensitivity to gibberellin and ethylene in the petioles of flooding-resistant *Rumex*. Many of the submergence reactions can also be induced by restricting the oxygen supply, suggesting that low-oxygen might be a triggering factor. The *Rumex* species we study represent various distinct communities. Thus, the ecophysiological phenomena observed in these model plants may explain processes and patterns in other species too and thus are interpretable at the riverside community level.

**Key words:** Ecophysiology, submergence, flooding, hormones, adaptation, nitrification, depth accommodation, adventitious rooting, *Rumex*.

### INTRODUCTION

Anthropogenically induced floods occur worldwide. Cutting of woodland, drainage of agricultural areas, canalization of rivers and urbanization are among the main causes of flooding of river areas. The irregular excess of water not only threatens the food supply of many human populations but also affects the natural vegetation in river plains. In western Europe, the Rhine is one of the many rivers exhibiting strongly fluctuating water levels that are partly the consequences of man-made dikes constructed to restrain the extent of flooding. The combination of different flooding regimes varying in frequency, intensity, duration and elevation, characterize flood plains as highly dynamic areas full of clearly distinguishable hydrological patterns that in turn give rise to high biodiversity (Van der Valk, 1981; Van der Valk and Welling, 1988; Blom *et al.*, 1990; Jongman, 1992; Boutin and Keddy, 1993).

Recording the frequencies and abundances of individual plant species provides insights into the process of vegetation succession that often is interrupted, especially close to the river bed. These records reveal a typical zonation within river plains ranging from early successional stages at the open sites situated along the edges of the river, via the mid successional stages of the less frequently flooded sites to the late successional habitats of the scarcely inundated parts of the area. Not only species composition but also life-history

characteristics and physiology of the co-occurring species will change during flood-dependent succession (Blom, 1990; Voeselek, 1990; Van der Sman, 1992; Van Groenendael *et al.*, 1993).

Our work aims to unravel the reactions of individual species to submergence, with the ultimate goal of gaining insight into the processes acting at the level of communities inhabiting the various zones in river plains. We use naturally occurring plants but results may also have relevance to responses of farm crops to flooding.

In this paper, two approaches are followed: First, in the 'more species—one habitat' approach, a number of species were chosen to screen the effects of various flooding regimes on survival and growth characteristics. These species were selected from higher and lower elevated sites in the mid-successional zone. In the literature, species from such mid-successional zones have tended to be overlooked; authors assuming that their life-history traits are less variable than in pioneer or late successional species (cf. Brown, 1992). The second or 'one genus—more habitats' approach aims to study a group of closely related species in more detail, especially with respect to their physiological characteristics. These model species were selected not only from the mid, but also from the early and late successional zones. All species under study, and their locations in the various zones in the river area, are presented in Fig. 1. Species from the late and mid successional zone are located at high to



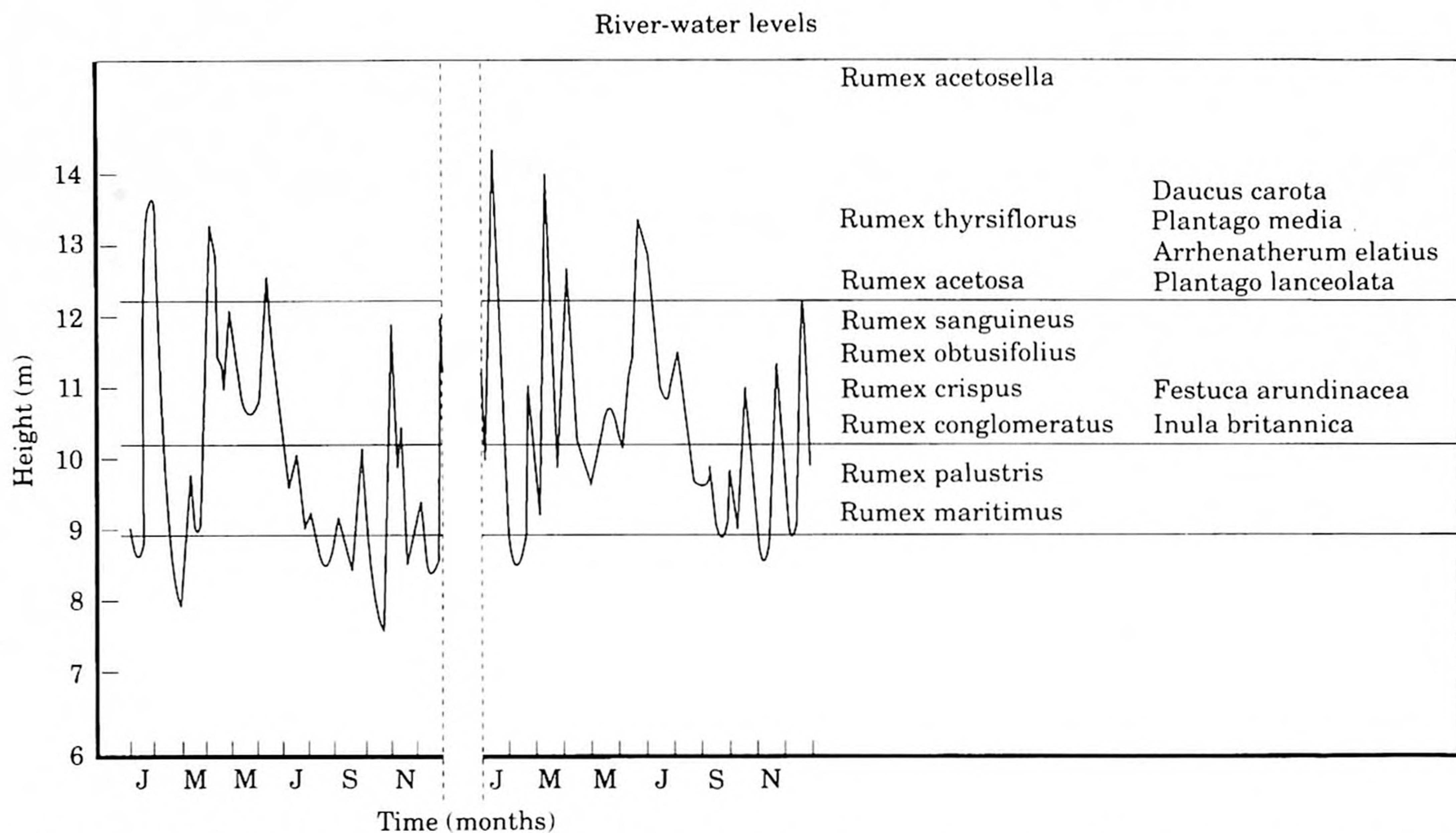


FIG. 1. Fluctuating water levels, in m above sea level, in the river Rhine and the subsequent zonation of the river area. Water levels were measured in the eastern part of the Netherlands in two randomly chosen years (1986, 1987). Zonation is indicated with the names of species under study.

intermediate elevated habitats; plants from the early successional zones colonize the borders along the river.

Up to the present, the following series of adaptative responses to submergence are known and have been researched: (a) Variation in life-history strategies at the levels of avoidance and tolerance of oxygen deprivation (Menges and Waller, 1983; Van der Sman, Joosten and Blom, 1993a; Van der Sman, Blom and Barendse, 1993b). (b) Changes in physiological and metabolic processes resulting in morphological adaptations leading to the avoidance of asphyxiation by submergence or soil flooding. These include the formation of new aerenchymatous roots during and after de-submergence or waterlogging of the soil (Justin and Armstrong, 1987; Laan *et al.*, 1989a; Laan, Clement and Blom, 1991b) and the accelerated growth, underwater, of stems and petioles (Voesenek and Blom, 1989a, b; Perik *et al.*, 1989; Voesenek *et al.*, 1990, 1992, 1993b).

This paper examines mechanisms of these adaptative reactions in order to understand more about the contribution of these responses to distribution, maintenance and survival of plants during the adverse conditions of the submergence period in the field.

Although the role of hormones in submerged or flooded plants has been extensively studied (e.g. Jackson, 1982, 1985; Jackson *et al.*, 1985, 1987; Voesenek *et al.*, 1990, 1993a, b; Brailsford *et al.*, 1993), attention will be paid to the many questions that are still open in ecological respects.

#### SURVIVAL, GROWTH AND RECOVERY OF PLANTS UNDER VARIOUS FLOODING REGIMES

Effects of submergence treatments on survival and growth were studied in *Daucus carota*, *Arrhenatherum elatius*, *Plantago media*, *Rumex acetosa*, and *P. lanceolata*. Each of

these species occurs on the higher elevated, seldom flooded sites of the mid-successional zones. Their reactions were compared with those of *Festuca arundinacea*, *Inula britannica* and *Rumex crispus* which grow in more frequently submerged parts of these zones in the river plain (Fig. 1). Eight-week-old plants were submerged in containers (width 1.85 m and depth 0.85 m) under greenhouse conditions. Duration of submergence was 1, 2 or 3 weeks for the species thought to be sensitive to flooding, and 3, 6 or 9 weeks for the more tolerant ones of the group. Submergence treatments were given in light to mimic clear-water conditions without large quantities of sediment. Submergence in the dark simulated flooding by silt-rich water. Survival and biomass production have been determined 1 week after the end of the submergence treatments. Recovery capacity was determined four weeks after 3 weeks of submergence.

Table 1 shows that presence and abundance of the species at the various elevations in the wild are in accordance with survival characteristics in greenhouse experiments. In the light, species from the higher sites survived submergence poorly, whereas the plants from lower habitats were better able to overcome the conditions. Most species were killed by submergence in the dark. In these severe conditions, only *Rumex crispus* survived, although for no longer than 6 weeks. Dry weight of the species from the higher elevations was strongly decreased, often to zero by submergence for 3 weeks or more (Tables 2 and 3). Species typical of the lower elevations retained green shoots even after 9 weeks of submergence. By the end of 9 weeks of submergence both *Plantago lanceolata* and *Inula britannica* retained a small amount of shoot and root biomass. However in contrast to most other species, *Inula* showed no significant decrease in root weight during 9 weeks submergence. An increase in shoot biomass during the first 3 weeks of submergence in clear water was found in *Rumex crispus*. This was the only species exhibiting shoot elongation when under water.



TABLE 1. Occurrence of species in the mid-successional zone of the river plain at Nijmegen (The Netherlands) and survival characteristics upon various periods of submergence in light or darkness

	Presence in % of samples (p) and mean cover (c in %) at different elevation levels						Survival % in light (l) and dark (d) at submergence in weeks					
	high		medium		low		3		6		9	
	p	c	p	c	p	c	l	d	l	d	l	d
<i>Daucus carota</i>	—	—	—	—	—	—	0	0	0	0	0	0
<i>Arrhenatherum elatius</i>	100	16.5	—	—	—	—	25	0	0	0	0	0
<i>Plantago media</i>	—	—	—	—	—	—	80	0	0	0	0	0
<i>Rumex acetosa</i>	89	9.0	—	—	—	—	100	0	60	0	0	0
<i>Plantago lanceolata</i>	89	3.0	33	0.5	20	0.5	100	0	100	0	20	0
<i>Festuca arundinacea</i>	—	—	66	3.0	30	2.0	100	0	100	0	100	0
<i>Inula britannica</i>	—	—	44	8.0	10	4.0	100	0	100	0	100	0
<i>Rumex crispus</i>	11	0.5	56	1.0	90	4.0	100	100	100	60	100	0

\*—, Not present.

TABLE 2. Root biomass (mg d. wt per plant) of several species selected from higher elevations (seldom flooded) and lower elevations (often flooded) in relation to submergence under greenhouse conditions in the light

	n	Submergence (weeks)				Recovery
		0	3	6	9	
Species from higher elevations						
<i>Daucus carota</i>	5	397 <sup>a</sup>	0	0	0	0
<i>Arrhenatherum elatius</i>	4	247 <sup>a</sup>	23 <sup>b</sup>	0	0	736 <sup>c</sup>
<i>Plantago media</i>	5	221 <sup>a</sup>	117 <sup>a</sup>	0	0	870 <sup>b</sup>
<i>Rumex acetosa</i>	5	790 <sup>a</sup>	324 <sup>b</sup>	113 <sup>bc</sup>	0	1058 <sup>d</sup>
<i>Plantago lanceolata</i>	5	246 <sup>a</sup>	124 <sup>b</sup>	50 <sup>bc</sup>	18 <sup>c</sup>	1126 <sup>d</sup>
Species from lower elevations						
<i>Inula britannica</i>	4	298 <sup>a</sup>	626 <sup>a</sup>	351 <sup>a</sup>	286 <sup>a</sup>	2998 <sup>b</sup>
<i>Rumex crispus</i>	5	2382 <sup>a</sup>	1917 <sup>ab</sup>	1863 <sup>ab</sup>	1623 <sup>b</sup>	5948 <sup>c</sup>
<i>Rumex crispus</i> (dark)*	5	2382 <sup>a</sup>	1584 <sup>b</sup>	868 <sup>ab</sup>	0	3685 <sup>c</sup>

For *Festuca arundinacea* no data on roots available.

The right-hand column (Recovery) shows biomass after 4 weeks recovery from 3 weeks of submergence.

Means in each horizontal line are different ( $P < 0.01$ ) if they carry different superscript letters (Bonferroni Test).Analysis was made after log<sub>e</sub> transformations.

\* All other species failed to survive dark submergence.

TABLE 3. Shoot biomass (mg d. wt per plant) of several species selected from higher elevations (seldom flooded) and lower elevations (often flooded) in relation to submergence under greenhouse conditions in the light

	n	Submergence in weeks				Recovery
		0	3	6	9	
Species from higher elevations						
<i>Daucus carota</i>	5	745 <sup>a</sup>	0	0	0	0
<i>Arrhenatherum elatius</i>	4	514 <sup>a</sup>	28 <sup>b</sup>	0	0	1142 <sup>c</sup>
<i>Plantago media</i>	5	674 <sup>a</sup>	825 <sup>a</sup>	0	0	2155 <sup>b</sup>
<i>Rumex acetosa</i>	5	1192 <sup>a</sup>	258 <sup>b</sup>	19 <sup>c</sup>	0	1329 <sup>d</sup>
<i>Plantago lanceolata</i>	5	812 <sup>a</sup>	636 <sup>ab</sup>	262 <sup>b</sup>	18 <sup>c</sup>	1816 <sup>d</sup>
Species from lower elevations						
<i>Festuca arundinacea</i>	5	1057 <sup>a</sup>	1120 <sup>a</sup>	391 <sup>b</sup>	148 <sup>c</sup>	2296 <sup>d</sup>
<i>Inula britannica</i>	4	469 <sup>a</sup>	872 <sup>a</sup>	568 <sup>a</sup>	462 <sup>a</sup>	2093 <sup>b</sup>
<i>Rumex crispus</i>	5	710 <sup>a</sup>	1350 <sup>b</sup>	1295 <sup>b</sup>	1209 <sup>b</sup>	2107 <sup>c</sup>
<i>Rumex crispus</i> (dark)*	5	710 <sup>a</sup>	433 <sup>b</sup>	6 <sup>c</sup>	0	1176 <sup>d</sup>

The right-hand column (Recovery) shows biomass after 4 weeks recovery from 3 weeks of submergence.

Means in each horizontal line are different ( $P < 0.01$ ) if they carry different superscript letters (Bonferroni Test).Analysis was made after log<sub>e</sub> transformations.

\* All other species failed to survive dark submergence.



Surprisingly, root biomass significantly decreased during the treatments. A limited amount of growth by this species appeared also during the first 3 weeks of submergence in darkness. Capacity to recover was considerable for all species tested except *Daucus carota* (Tables 2 and 3). By and large, after 4 weeks of recovery, biomass was twice or more than twice that at the start of the treatments.

*Rumex crispus* exhibited the most pronounced adaptive responses to flooding of the nine species tested. In contrast, *Daucus*, *Arrhenatherum* and *Plantago media* especially, were extremely susceptible to injury from submergence. Among the species from the elevated zone, *Rumex acetosa* was the only species that resisted flooding to any great extent.

These screening experiments provide a first indication of the adaptive behaviour of species upon submergence. To elucidate in more detail the mechanisms of the adaptations in roots and shoots, *Rumex acetosa* and *Rumex crispus* were compared with others in the same genus that are found commonly in other successional stages. They each serve as model species for plant communities being exposed to different submergence conditions.

#### ADVENTITIOUS ROOT FORMATION, FUNCTIONALITY OF THE RENEWED ROOT SYSTEM IN *RUMEX* AND RESTORATION OF PHYSIOLOGICAL PROCESSES

An adaptation of many plant species to flooding is the accelerated and increased formation of adventitious roots. These roots, which develop particularly well when plants are submerged, often contain more aerenchyma than the primary roots (Justin and Armstrong, 1987; Laan *et al.*, 1989a; Smirnoff and Crawford, 1983). The genus *Rumex* shows a wide variation in the extent of adventitious root formation. Species from low elevations along river banks [and thus often flooded for long periods of time (see Fig. 1)] are usually well adapted to these floods. Experiments showed they develop large numbers of adventitious roots when waterlogged (Table 4). In contrast, adventitious rooting of species that grow on seldom flooded river habitats was poor.

The soil redox potential can decrease within a few hours of waterlogging to values (less than 330 mV; Fig. 2) that come close to indicating a complete absence of oxygen. Therefore, the rooting response of *Rumex* plants to waterlogging must be fast to avoid strong negative effects on plant viability. Within 2 d of waterlogging the soil, adventitious roots were found at the base of the shoots of *R. palustris*, and the number increased markedly with time (Fig. 3). These later roots developed mainly from the basal parts of the shoot and from the upper part of the tap root. Adventitious rooting by *Rumex* can also be induced by transferring hydroponically grown plants to a stagnant hypoxic agar solution (Fig. 4; Laan *et al.*, 1989a). Adventitious root formation seems to be triggered by low oxygen concentrations in the root system.

The large gas-filled spaces in adventitious roots provide a route for strong oxygen diffusion from the emerged shoot to the root tips. This so called 'internal aeration' can in some

cases maintain maximum aerobic respiration for limited periods in roots growing in anoxic flooded soils (Armstrong, 1979; Gaynard and Armstrong, 1987; Laan *et al.*, 1990). When the shoot is still in contact with the atmosphere, oxygen can reach the root via the shoot through such aerenchymatous tissue. When the plant is completely submerged, oxygen may become available from underwater photosynthesis (Laan and Blom, 1990; Voesenek *et al.*, 1993a). In most plants showing internal aeration, not all oxygen reaches the root tip. Some of the oxygen is consumed by respiration en route. Some diffuses out of the root radially, thereby re-oxidizing the first thin layer around the roots in the otherwise anaerobic soil (Smits *et al.*, 1990; Laan *et al.*, 1989b). The benefit of this phenomenon to the plant is the immobilization of resulting metal ions such as  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ , which become toxic for the plant when in its chemically reduced and thus insoluble form (Bienfait, 1989; Laan *et al.*, 1989b; Ernst, 1990; Laan, Smolders and Blom, 1991a). Furthermore, processes such as ion uptake by root cells (Grosse and Meyer, 1992) and active bacterial nitrification (Blacqui re, 1986; Laanbroek, 1990; Both, Gerards and Laanbroek, 1992) may take place. In contrast, chemically reduced soils possess no oxygen for respiration (Ponnamperuma, 1984); important oxygen-dependent microbial processes are inhibited and toxic compounds from anaerobic metabolism may accumulate.

Nitrification is one of the most important microbial processes with regard to plant nutrition. It generates available nitrate for the plant and is dependent on oxygen. In theory, a plant with aerenchymatous roots showing radial oxygen loss should be able to maintain nitrifying activity in the soil surrounding its roots. This was tested in waterlogged and drained soils by monitoring *Rumex thyrsiflorus*, a species unable to produce aerenchymatous roots and *R. palustris*, a species that readily produces aerenchymatous roots (Table 4) and showing radial oxygen loss from these roots (Laan *et al.*, 1989a). Plants were grown for 63 d in river sand with a small amount of pasture soil serving as an inoculum for nitrifying bacteria. Nitrogen was provided as  $\text{NH}_4^+$ . In drained pots of both species, redox potentials exceeded 450 mV throughout the experiment, indicating the presence of free oxygen. In the waterlogged pots with *R. thyrsiflorus*, the redox potential dropped upon waterlogging and remained stable at 250 mV. At this level virtually no free oxygen would be present. In waterlogged pots with *R. palustris*, an initial decrease in redox potential was followed by a stabilizing at 350–390 mV, indicating entry of oxygen into the soil. The status of the nitrifying population was checked by measuring the potential nitrifying activity of the soil. This was achieved by incubating soil in a medium containing  $\text{N}_2$  for several hours and analysing the amounts of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  produced. In the waterlogged pots with *R. thyrsiflorus* the potential nitrification activity decreased to only 3% of that in well-drained pots after 6 weeks of soil waterlogging (Table 5). At the same time waterlogged pots of *R. palustris* still showed a considerable potential nitrification activity, maintained at about 30% of the activity present in the drained soil. Therefore, we conclude that in the soil with *R. palustris* two vital ingredients for nitrification, oxygen and an active



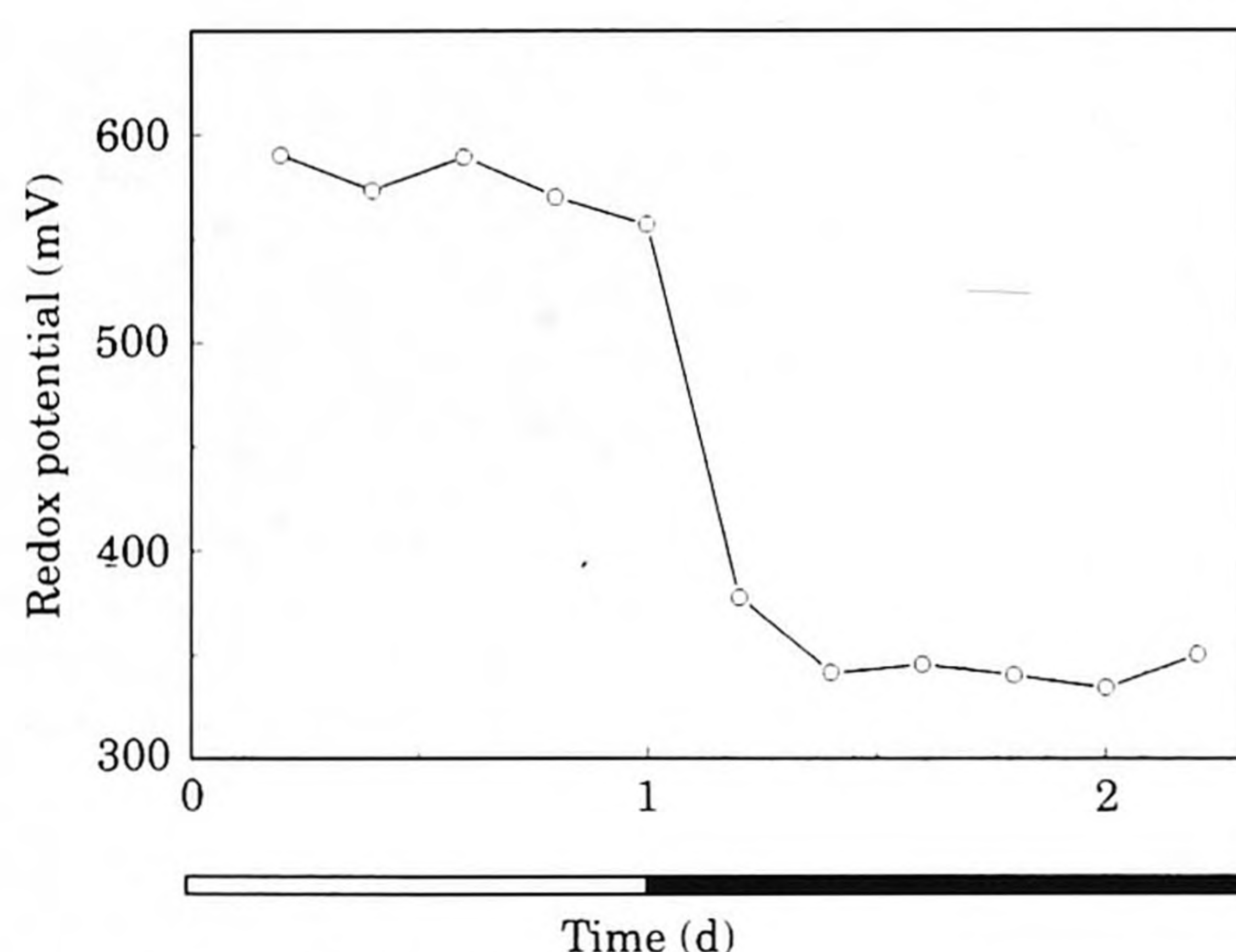


FIG. 2. Redox potential at a depth of 5 cm in a clay-sand mixture (without plants) which was waterlogged on day 1 (representative result of four experiments).

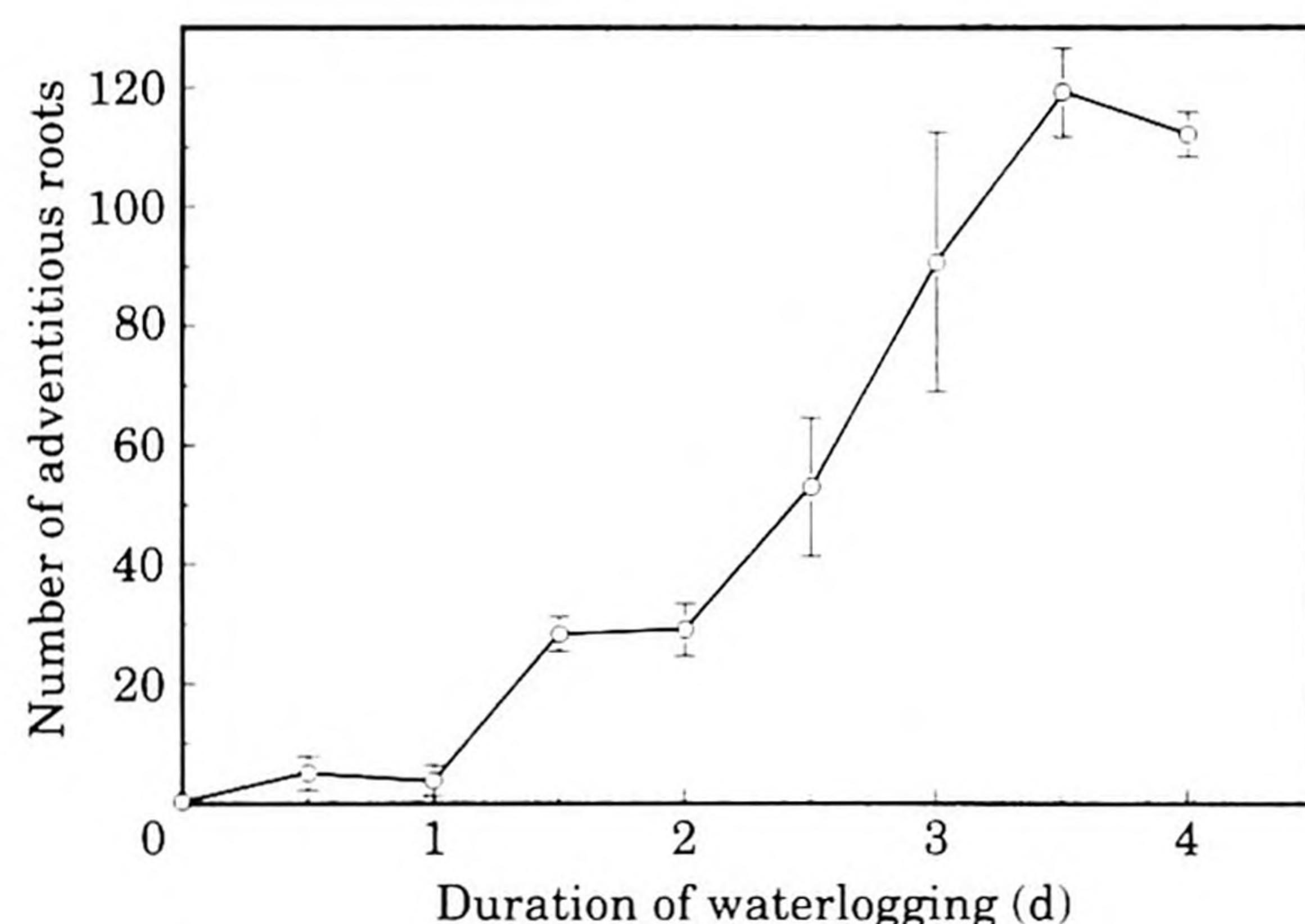


FIG. 3. Adventitious root formation by 6-week-old plants of *Rumex palustris* during waterlogging. Vertical bars represent s.e. above and below each mean of three replicates.

nitrifying population, were still present. The third factor,  $\text{NH}_4^+$ , was constantly provided with the nutrient solution. By measuring the nitrate reductase activity in the leaves, we

TABLE 4. Adventitious root formation by six *Rumex* species after 4 and 16 d of soil waterlogging (mean of 3 replicates  $\pm$  s.e.). The species occur in the field along a flooding-frequency gradient from seldom flooded (*R. thyrsiflorus*) to regularly waterlogged or submerged (*R. palustris*)

Natural habitat	Species	Number of adventitious roots per plant	
		after 4 d	after 16 d
Dry ↓ Wet	<i>R. thyrsiflorus</i>	$8.0 \pm 2.3$	$37.3 \pm 6.0$
	<i>R. acetosa</i>	$22.8 \pm 5.8$	$58.3 \pm 5.0$
	<i>R. obtusifolius</i>	$16.3 \pm 1.7$	$61.3 \pm 1.7$
	<i>R. crispus</i>	$17.8 \pm 2.8$	$61.3 \pm 4.2$
	<i>R. sanguineus</i>	$23.3 \pm 3.4$	$121.0 \pm 6.1$
	<i>R. palustris</i>	$49.8 \pm 1.7$	$130.5 \pm 9.8$

TABLE 5. Nitrifying capacities ( $\text{nmol NO}_2^- + \text{NO}_3^- \text{ g}^{-1}$  dry soil  $\text{h}^{-1}$ ) of drained and waterlogged soils with either *Rumex thyrsiflorus* or *R. palustris* after being provided with  $\text{NH}_4^+$  over 9 weeks

Soil	<i>R. thyrsiflorus</i>	<i>R. palustris</i>
Drained	$95.28^*$	$183.69^*$
Waterlogged	3.48	55.56

Means of five replicates.

\* A significant difference from waterlogged treatments ( $P \leq 0.05$ , Mann-Whitney  $U$ -test).

checked that  $\text{NO}_3^-$  had indeed become available to the plants of *R. palustris* in the waterlogged pots. This parameter has been found to be a good indicator for  $\text{NO}_3^-$  availability in this species; nitrate reductase activity in the leaves of plants treated for 6 weeks with  $\text{NO}_3^-$  or a mixture of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  being higher compared to plants provided with only  $\text{NH}_4^+$  (Table 6). In support of the idea that *R. palustris* was supplied with  $\text{NO}_3^-$  when waterlogged, nitrate reductase

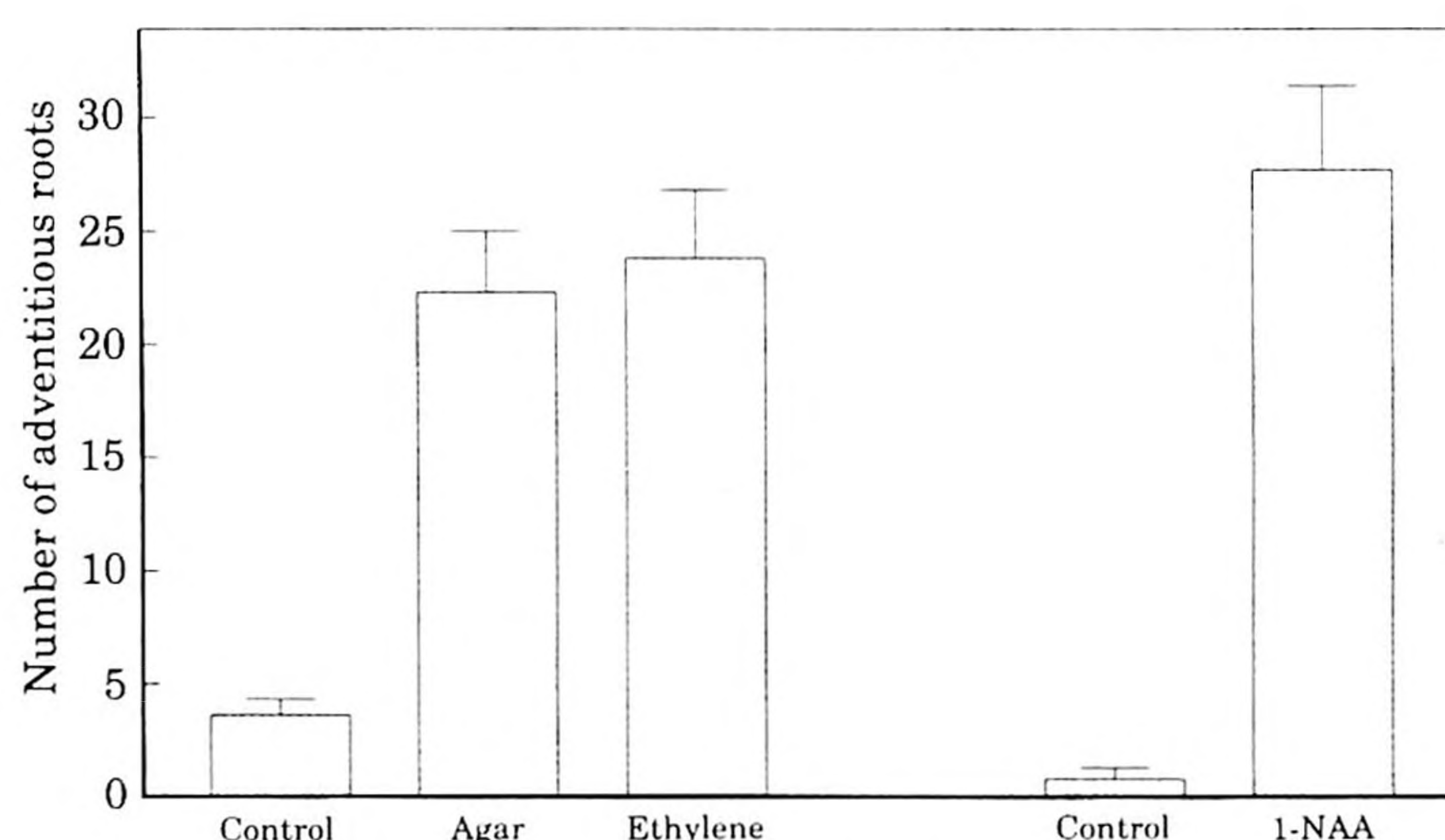


FIG. 4. Adventitious root formation by 4-week-old plants of *Rumex palustris* 7 d after treatment of the root system with either hypoxic stagnant agar solution (0.05% in nutrient solution) or nutrient solution containing 2.5 ppm ethylene. In a separate experiment, some plants were treated with 0.25  $\mu\text{mol}$  1-NAA to the shoots.  $n = 10$  (for agar or ethylene treatments) or  $n = 8$  (NAA treatment). Vertical bars show s.e. above each mean.



activity in leaves of waterlogged plants was no less than that from freely drained plants. But, the nitrate reductase activity of waterlogged pots with *R. thyrsiflorus* were very much decreased, compared to well-drained plants and even when compared to plants provided only with  $\text{NH}_4^+$ .

#### THE ROLE OF HORMONES AND LOW OXYGEN IN THE ADAPTATIVE RESPONSES OF *RUMEX* SHOOTS

The gaseous growth substance ethylene promotes not only adventitious rooting and aerenchyma formation (Drew, Jackson and Gifford, 1979) but also shoot elongation (Musgrave, Jackson and Ling, 1972), which is another well known adaptive response upon submergence in many wetland species (Yamamoto and Kozlowski, 1987a,b; Yamamoto, Kozlowski and Wolter, 1987; Ridge, 1987; Voesenek and Blom, 1989a,b; Laan and Blom, 1990; Schwegler and Brändle, 1991; Van der Sman *et al.*, 1991; Crawford, 1992). Figure 5 illustrates the elongation responses of differently aged leaves in two *Rumex* species. Enhanced leaf elongation was observed in both leaves of *R. palustris*. In *R. acetosella*, however, no differences in leaf elongation were detected between submerged and non-submerged plants.

Possibly, other plant hormones also play a key role in the chain of reactions upon submergence and hypoxia, leading to a differential response towards ethylene within plants (Yamamoto and Kozlowski, 1987c; Jackson, 1990). Involvement of indole-3-acetic acid (IAA), gibberellin (GA) and abscisic acid (ABA) in depth accommodation has been described for a number of amphibious species i.e. *Callitriche platycarpa*, *Ranunculus sceleratus* and *Oryza sativa* (Musgrave *et al.*, 1972; Musgrave and Walters, 1973; Samarakoon and Horton, 1983; Suge, 1985; Hoffmann-Benning and Kende, 1992). However, for species of the genus *Rumex*, little is known of the role of other plant hormones other than ethylene in the process of depth accommodation. The next part of this section will describe the role of ethylene, low oxygen, auxin and gibberellin in the submergence response of the shoots of *R. acetosella*, *R. acetosa* and *R. palustris* (see Fig. 1 for position of the species in the flood gradient).

##### Ethylene

We assessed the question of whether or not the marked difference in the ability of the flood-tolerant *R. palustris* and the intolerant *R. acetosella* to elongate rapidly when submerged is caused by a difference in ethylene production or by a variation in internal concentration. Under experimental conditions, the relationships between ethylene entrapment during submergence and the release after de-submergence were studied in relation to the internal gas volumes of the plants. Whole plants just starting the development of their fifth leaf were used (*R. palustris*, 24 d-old; *R. acetosella*, 27 d-old; for growth conditions see Voesenek and Blom, 1989b). Ethylene release in the air phase, was measured in a continuous flow system using a

laser-driven intracavity photoacoustic detection system (Voesenek *et al.*, 1990). Ethylene in the air above submerged *R. acetosella* was below the detection limit of the ultra sensitive photoacoustic laser-driven ethylene detector (approx.  $0.05 \text{ nl l}^{-1}$ ), indicating a very low ethylene production level in this species (Table 7). In contrast, the quantities found for *R. palustris* were somewhat greater. The amount of entrapped ethylene (nl) was measured by the same detection system. Immediately after de-submergence, ethylene accumulating during 24 h of total submergence is quickly released from the plants, and this can be measured by the photoacoustic method. Just before de-submergence, glass cuvettes each containing a plant were closed, wrapped in aluminium foil and connected to the continuous flow system of pure nitrogen. This procedure removed all oxygen from the atmosphere of the cuvettes and prevented the generation of oxygen in the plants by photosynthesis. Under these conditions ethylene production after de-submergence is completely prevented (see also Voesenek *et al.*, 1993b). The internal air volume (ml) of the same individuals was determined using a modified pycnometer method (Jensen *et al.*, 1969). All gas was removed from the plants by submersing them in water in a dessiccator to which a vacuum of 10 kPa was applied. Endogenous ethylene concentration after 24 h of submergence ( $\text{nl ml}^{-1}$ ) was obtained by dividing the amount of entrapped ethylene (nl) released by the internal gas volume (ml) of the individual plant. Due to rounding errors, dividing the mean amount of entrapped ethylene by the mean internal air volume does not give exactly the same value as the endogenous ethylene concentration given in Table 7.

In drained *R. palustris* and *R. acetosa* (very comparable to *R. acetosella*) an endogenous ethylene concentration of  $0.05 \text{ nl ml}^{-1}$  was found using gas chromatography and a refined vacuum gas extraction technique (Voesenek *et al.*, 1993b). Results presented in Table 7 also show the extremely small amount of entrapped ethylene after 24 h of submergence in *R. acetosella*. However, submergence did result in a 90-fold increase in the endogenous ethylene concentration due to the very small internal gas volume of this species. In contrast *R. palustris* produced relatively more ethylene in terms of total amount (volume). However, this led to an internal ethylene concentration which was only twice as high as in *R. acetosella*, because of the much larger internal gas volume of this species.

##### Low oxygen

Submergence not only leads to inhibition of gas exchange between the open air and the soil but also to oxygen depletion. An experiment was designed in which two oxygen levels were applied to investigate to what extent low oxygen levels influence the adaptation by the shoot of the submerged plant. To gain insight into the combined action of low oxygen availability and increased ethylene concentrations, plants growing under hypoxic conditions were treated with ethylene, its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) and an inhibitor of ethylene production aminoethoxyvinylglycine (AVG).



TABLE 6. Nitrate reductase activity in leaves of *Rumex thyrsiflorus* and *R. palustris* plants provided with  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  or both together during 5 weeks ( $n = 3$ ), and when grown in drained and waterlogged soil ( $n = 5$ ) for 6 weeks

	Nitrate reductase activity ( $\mu\text{mol g}^{-1} \text{ d. wt h}^{-1}$ )	
	<i>R. thyrsiflorus</i>	<i>R. palustris</i>
$\text{NO}_3^-$	12.14 <sup>a</sup>	15.43 <sup>a</sup>
$\text{NO}_3^-/\text{NH}_4^+$	10.00 <sup>a</sup>	11.47 <sup>ab</sup>
$\text{NH}_4^+$	2.86 <sup>b</sup>	2.17 <sup>c</sup>
Drained	6.02 <sup>a</sup>	8.08 <sup>b</sup>
Waterlogged	2.66 <sup>b</sup>	8.91 <sup>b</sup>

Means in each row with different superscript letters are significantly different ( $P \leq 0.05$ , Mann-Whitney  $U$ -test).

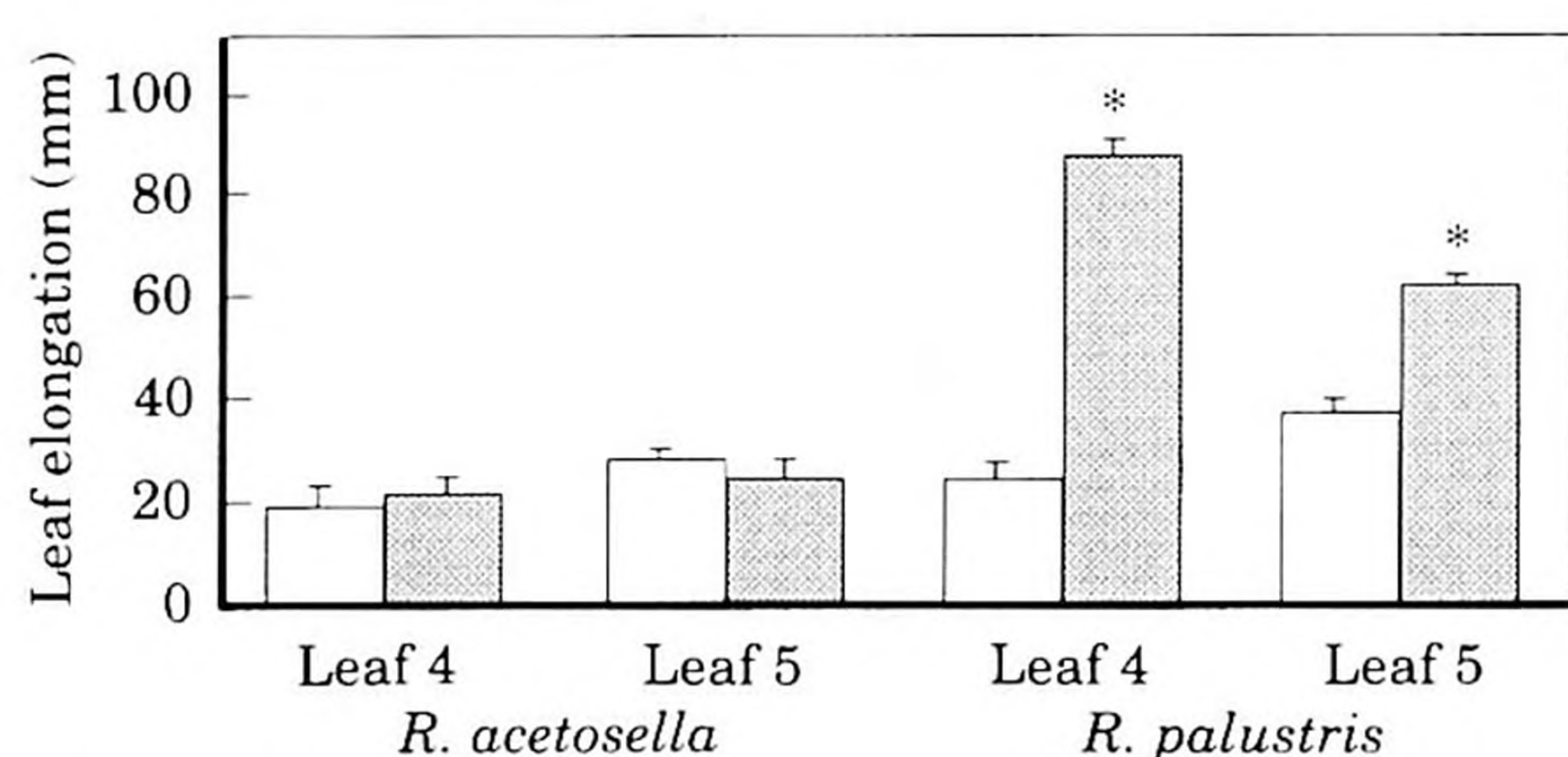


FIG. 5. Leaf (lamina + petiole) elongation of intact plants of *Rumex palustris* (24-d-old) and *R. acetosella* (27-d-old) after 7 d complete submergence. Control plants (□) were kept drained. Fifth leaf was just emerging at the time treatments began (for growth conditions see Voisenek and Blom, 1989b). Means marked (\*) are significantly different from their control (Tukey test;  $P < 0.05$ ). Vertical bars show standard error above each mean ( $n = 10$ ).

TABLE 7. Ethylene release and endogenous ethylene concentrations of two *Rumex* species after 24 h of total submergence

	<i>R. acetosella</i>	<i>R. palustris</i>
$\text{C}_2\text{H}_4$ -release ( $\text{nl g d. wt h}^{-1}$ )	0	$4.74 \pm 0.75$
Entrapped $\text{C}_2\text{H}_4$ (nl)	$0.13 \pm 0.03$	$2.20 \pm 0.08$
Internal gas volume (ml)	$0.031 \pm 0.05$	$0.247 \pm 0.034$
$\text{C}_2\text{H}_4$ -concentration ( $\text{nl ml}^{-1}$ )	$4.47 \pm 1.15$	$9.21 \pm 1.13$

Means of three replicates, with standard errors.

In *R. palustris*, petiole elongation was enhanced under a sub-ambient oxygen concentration of 3% in both the absence and presence of ethylene (Fig. 6). This low-oxygen effect was apparently acting via ethylene since the addition of the ethylene biosynthesis inhibitor (AVG) prevented the promotion of petiole extension. Specificity of AVG was tested by addition of the immediate precursor of ethylene (ACC) which overcame the AVG effect and returned elongation growth back to that in 3% oxygen in the absence of AVG (Fig. 7).

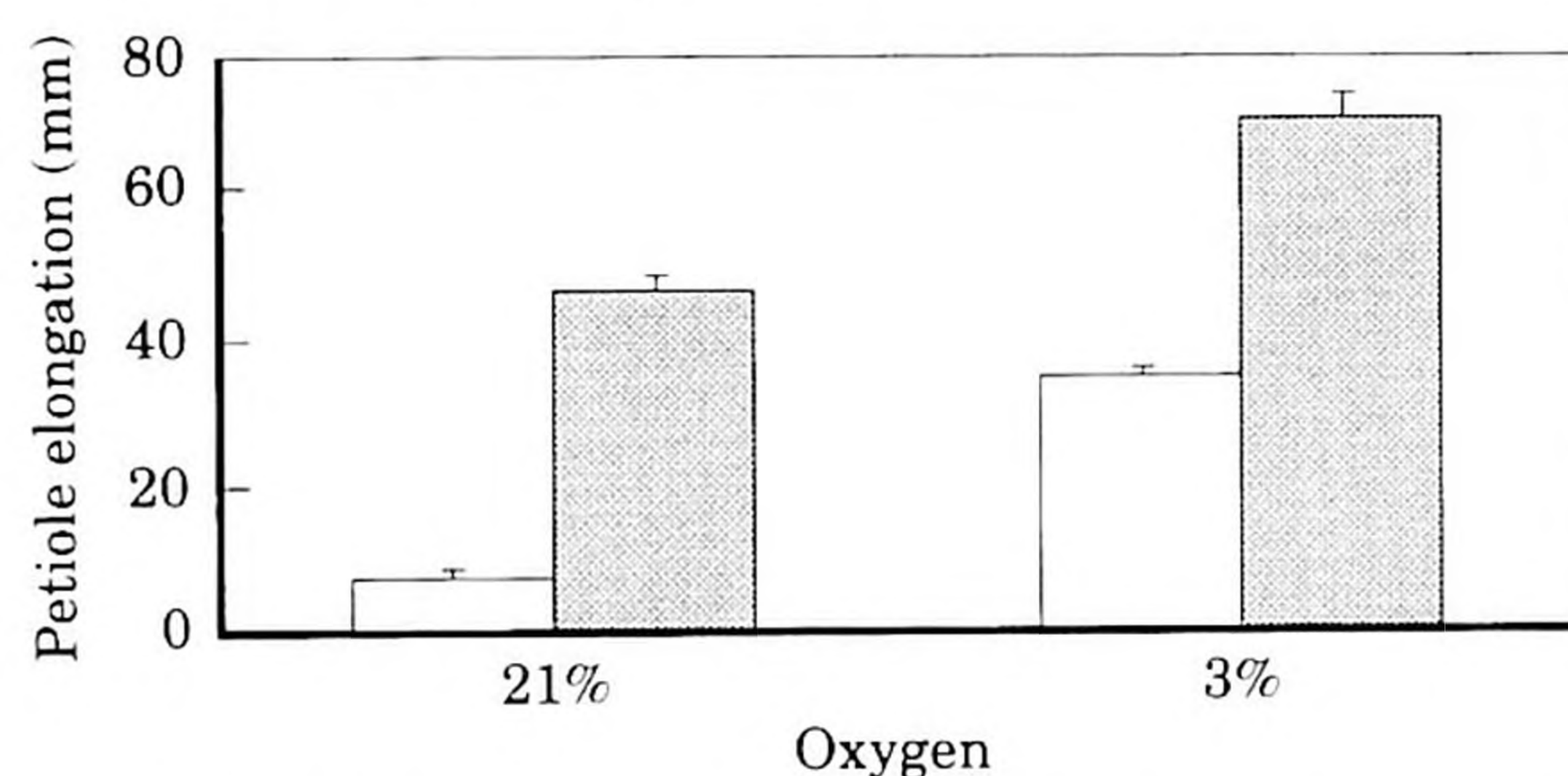


FIG. 6. Petiole elongation by 26-d-old intact *Rumex palustris* plants during exposure for 4 d to continuous flows ( $1 \text{ l h}^{-1}$ ) of 21 or 3% oxygen and  $< 0.01$  (□) or  $5.0 \text{ nl ml}^{-1}$  (■) ethylene. Means of 18 replicates, with vertical lines showing 1 s.e. above each mean.

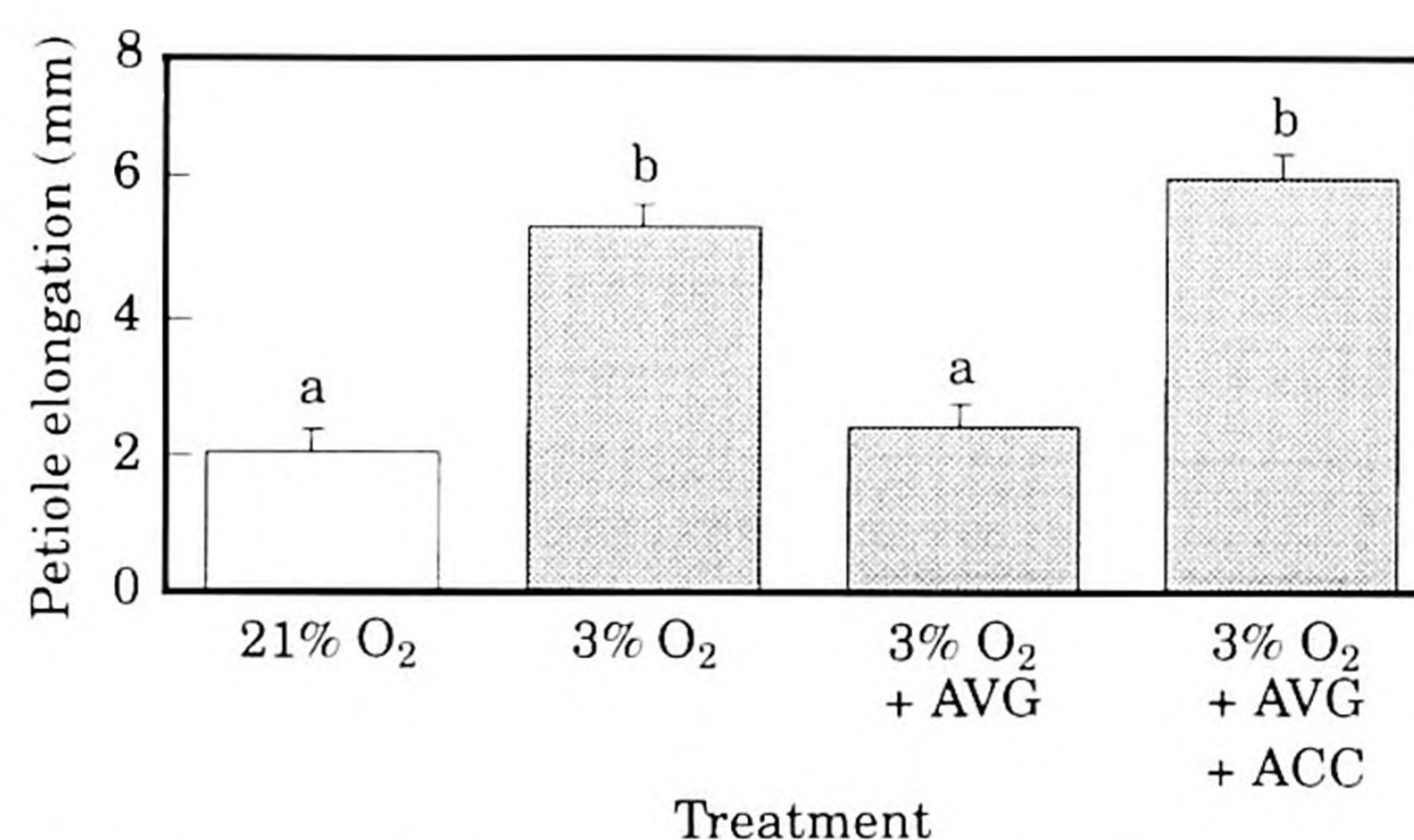


FIG. 7. Elongation of excised petioles of *Rumex palustris* during a 48 h exposure to 21 or 3% oxygen, 0.05 mM aminoethoxyvinylglycine (AVG) and 1 mM 1-aminocyclopropane-1-carboxylic acid (ACC). Means with the same letter are not significantly different (Tukey test;  $\alpha < 0.05$ ). The experiment was performed with isolated petioles with the leaf blade and 1 cm of tap root still attached. The petiole used was from the youngest leaf of 26-d-old plants (for growth conditions see Voisenek and Blom, 1989b). AVG and ACC were applied continuously to the tap-root in 10 mM phosphate buffer (pH 6.0).

#### Auxin and gibberellin

As shown in Table 7, the observed differential shoot responses of various *Rumex* species upon inundation cannot be explained solely by an increase in endogenous ethylene. Both the flooding intolerant *R. acetosa* or *R. acetosella* and the tolerant *R. palustris* have elevated internal ethylene concentrations. However, upon submergence or external application of ethylene, shoot elongation by *R. palustris* is promoted while that of *R. acetosa* and *R. acetosella* becomes restricted (Table 7; Voisenek and Blom, 1989a, b; Voisenek et al., 1990). Apparently, growth promoters other than ethylene, possibly auxin and gibberellins, are involved in the adaptation reaction. To test the involvement of auxin, plants of *R. palustris* were pretreated with the auxin-transport inhibitor *N*-1-naphthylphthalamic acid (NPA) before submergence. However, NPA had no effect on petiole elongation. Comparable results were found for submergence of plants in the synthetic auxin 1-naphthaleneacetic acid (NAA), or 2-NAA (Fig. 8). Detailed analyses of variance on treatments within groups showed no effects of NPA or NAA except for 1 mM NAA which strongly inhibited growth. This was accompanied by an



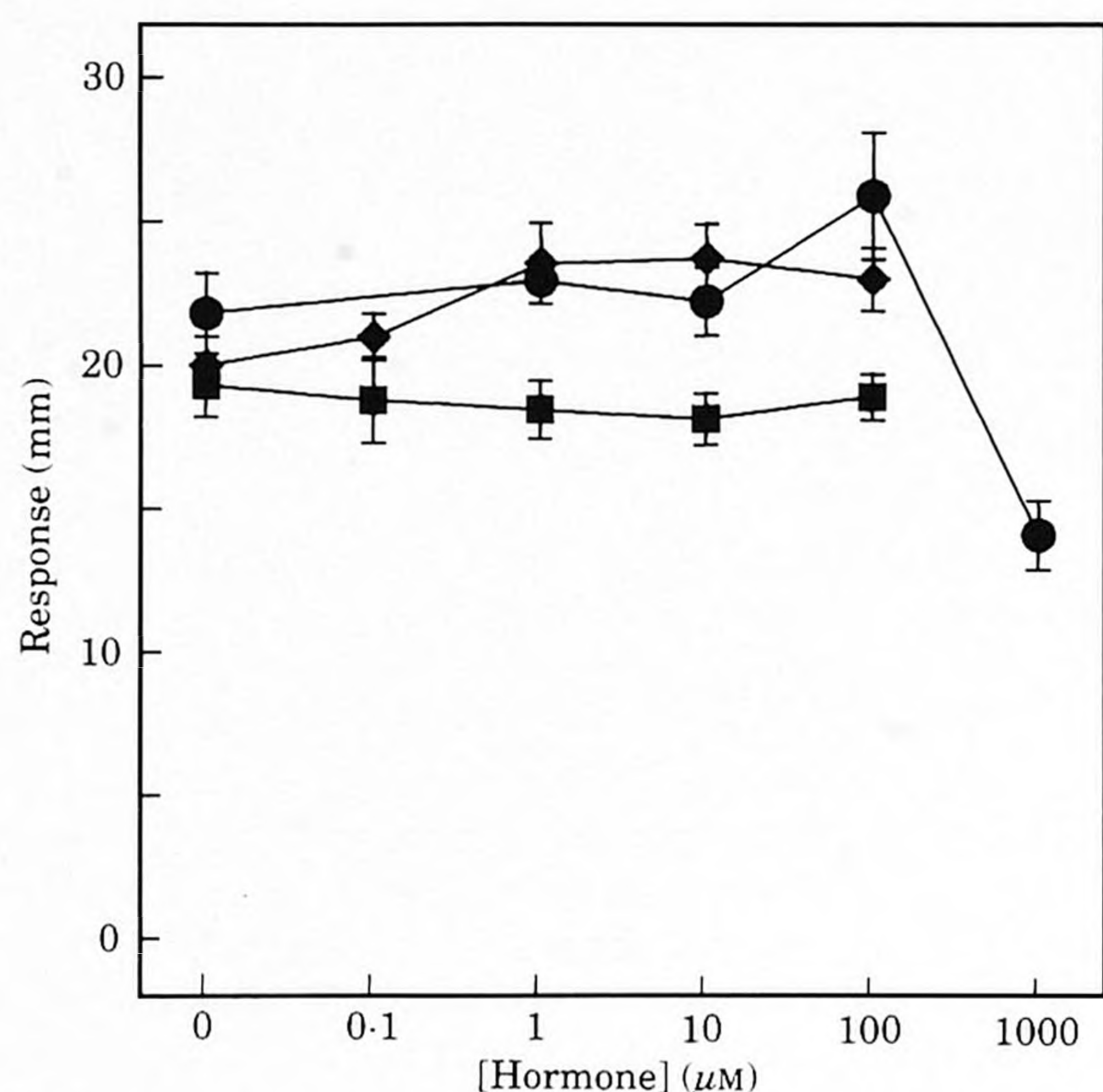


FIG. 8. Growth response of petioles of intact *Rumex palustris* plants to submergence in solutions of (●) NAA, (▲) NPA or (■) 2-NAA applied in 10 mM phosphate buffer (pH = 6.0). Plants were submerged for 48 h, growth of the petioles of the youngest leaves was measured. Means of 10 replicates. Vertical lines show s.e. above and below each mean.

anomalously rigorous twisting of the petioles thus considered non physiological. Submergence in indole acetic acid (IAA) gave comparable results as NAA (results not shown). Data obtained with isolated petioles, with or without aging prior to treatments gave similar results to intact plants.

Paclobutrazol pretreatment of submerged plants caused

TABLE 8. Effect of the gibberellin-biosynthesis inhibitor paclobutrazol, and of gibberellic acid ( $GA_3$ ) on the extension of petioles of *Rumex palustris*

	Elongation (mm 2 d <sup>-1</sup> )		
	Drained	Submerged	Submerged + $GA_3$
No paclobutrazol	18.1 <sup>a</sup>	33.8 <sup>c</sup>	33.5 <sup>c</sup>
Paclobutrazol	—	17.2 <sup>a</sup>	25.8 <sup>b</sup>

Means of 10 replicates.

Means with different superscript letters are significantly different from plants grown under drained conditions with no paclobutrazol and  $GA_3$ . ( $P < 0.01$ , Tukey's test).

an inhibition of petiole elongation (Table 8). This inhibition was partially overcome by the application of 0.01 mM  $GA_3$ . Dose-response curves obtained after paclobutrazol and  $GA_3$  treatment of both drained and submerged plants differed markedly (Fig. 9). Submergence enhanced the response to exogenously applied  $GA_3$  by steepening the slope of the response to concentrations between 0.01–0.0001 mM, and raised the maximal response.

## DISCUSSION AND CONCLUSIONS

We believe that our attempts to bridge the gap between physiology and ecology will clarify relationships between adaptive responses of individual plants and species distribution in the real world. The use of model species that serve as indicators for larger groups of plants allows an extrapolation of results to the level of plant communities. Later screening of adaptation reactions in other than model species can then be expected to validate the results obtained

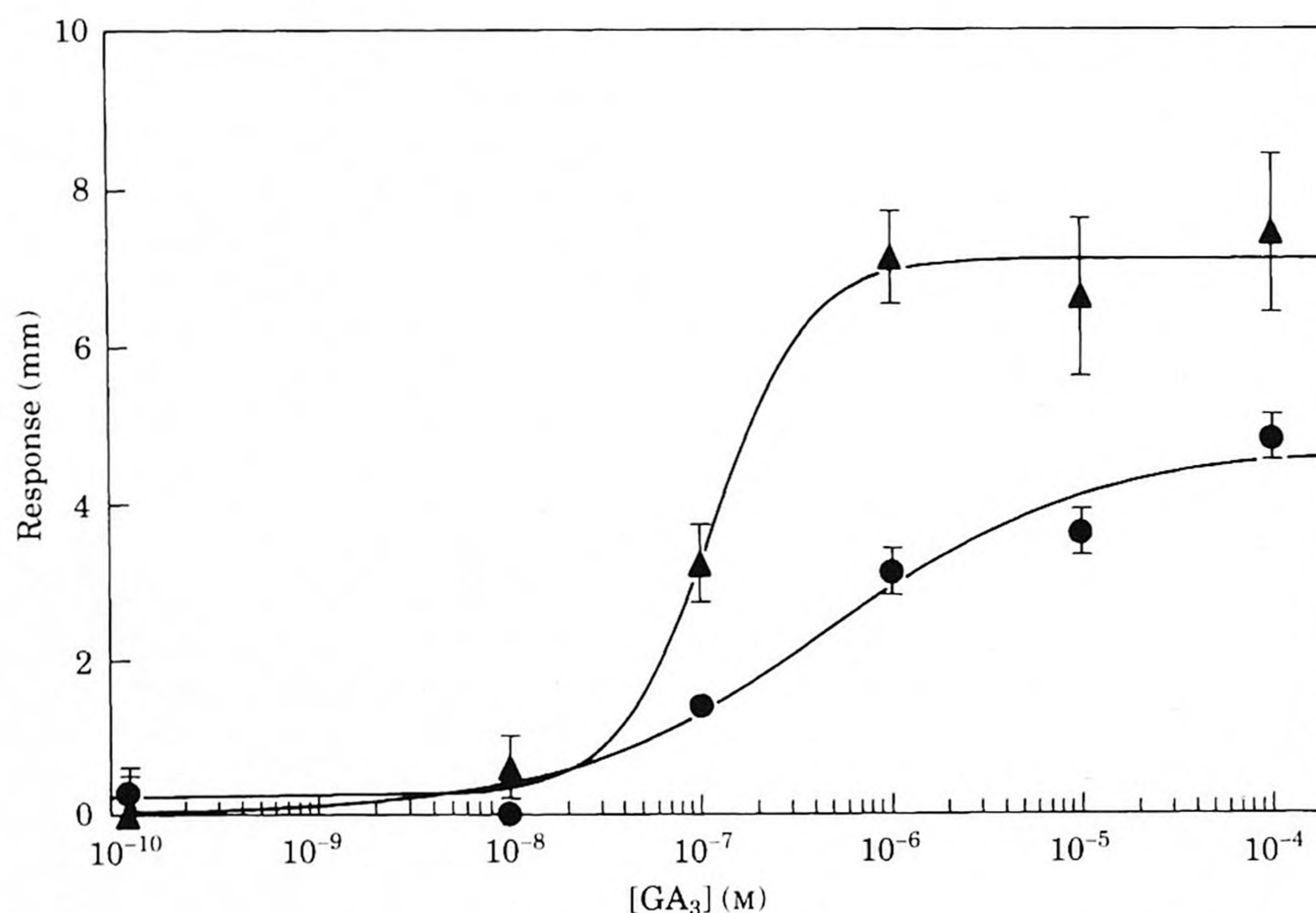


FIG. 9. Petiole elongation dose-response curves of *Rumex palustris* to gibberellin. Plants were pretreated with paclobutrazol (10 ml, 0.1 mM) and  $GA_3$  (10 ml, 0.0–0.1 mM) respectively 4 d and 3 h prior to experiment. Forty eight hour experimental treatments were: (a) drained (●) and (b) submerged (▲). Points are the mean ( $\pm$  s.e.) of 10 plants. Curves were fitted on individual points with a Michaelis-Menten equation (for drained and submerged conditions respectively:  $r^2$ : 0.99 and 0.97).



with the indicator plants. Plants from the early successional zones in the river area exhibit strategies characterized by fast turnover rates (Van der Sman, Van Tongeren and Blom, 1988; Blom *et al.*, 1990; Blom, Voesenek and Van der Sman, 1993). During the relatively short periods of water deprivation between successive floods, plants of these species (e.g., *Chenopodium rubrum*) very quickly complete their life cycle and produce seeds that will survive the next flood (Van der Sman *et al.*, 1991, 1993a, b). Plants from the late-successional zones (e.g. *Rumex acetosa*) grow in densely covered habitats, are highly competitive and perennial (Blom, 1990; Voesenek, 1990). Species from mid-successional zones may be exposed to both flooding and competition. These plants adopt a rather opportunistic strategy. Successful species here cope with the adverse conditions of submergence by retaining much root and shoot biomass that can re-grow vigorously when desubmerged (Tables 2 and 3). After only one period of submergence some species from the elevated habitats are still able to recover, but field observations have demonstrated that these species, in contrast to plants occurring in lower sites, totally collapse if submerged more than once during the growing season.

Resistant plants tolerate submergence by their abilities to accelerate shoot extension that brings the upper leaves back into contact with the aerial environment. These same plants also form new adventitious roots and an aerenchymatous channel system to provide oxygen to the roots and rhizosphere. Due to aerenchyma formation, species such as *R. palustris*, show radial oxygen loss and maintain an oxidized rhizosphere with an active nitrifying population of bacteria that provides the plant with nitrate that appears to be taken up as judged by the high nitrate reductase activity. There are some limitations to the preservation of nitrification in waterlogged soils. When  $\text{NH}_4^+$  is in short supply the plant and other soil microorganisms have proven to be better competitors than plants for the  $\text{NH}_4^+$ , thus suppressing the nitrifying population (Engelaar *et al.*, 1991). Furthermore, when oxygen deprivation in the soil is combined with a small soil pore diameter (i.e., compacted soil), aerenchyma formation and radial oxygen loss are inhibited (Engelaar *et al.*, 1993). Under these conditions no oxygen will be present in the soil and nitrification becomes inhibited.

It has been known for several years that ethylene is the most important hormone involved in acclimation and adaptation mechanisms towards submergence (Osborne, 1984). This reflects the intimate relationship between submergence and ethylene levels in plants. Yet, it is also clear that other growth substances must be involved. It is widely recognised that plant hormones work in combination. Applying auxin to the shoot of a *R. palustris* plant resulted in the same number of adventitious roots as hypoxia of the root system did (Fig. 4). Remarkably, the same response was evoked by supplying the primary root system with low concentrations of ethylene (Fig. 4). The oxygen status of the roots was in both cases sufficient for normal aerobic respiration. These promoting effects of auxin and ethylene are commonly found with respect to adventitious root formation in soft- and hard-wooded cuttings (e.g. Gurumurti, Gupta and Kumar, 1985; Robbins *et al.*, 1985;

Bollmark and Eliasson, 1990; Nordström and Eliasson, 1991; Selby, Kennedy and Harvey, 1992).

In contrast to the rooting response, auxin seems not to be involved in petiole elongation of *R. palustris*. Application of NPA, or IAA and 2-NAA had little effect (Fig. 8). Thus, this species contrasts with *Regnellidium diphyllum*, *Ranunculus sceleratus* and *Nymphoides peltata*, in which auxin is required for ethylene action (Walters and Osborne, 1979; Horton and Samarakoon, 1982; Malone and Ridge, 1983). A combination of ethylene and auxin treatment of three aquatic plants, *Regnellidium diphyllum*, *Hydrocharis morsus-ranae* and *Ranunculus sceleratus* showed that ethylene and auxin effects are additive (Cookson and Osborne, 1978; Smulders and Horton, 1991) and both enhance elongation. Neither phenomenon was found in *R. palustris*. Apparently, not all ethylene mediated growth is auxin dependent. This emphasizes the need for comparative studies between *R. palustris* and, for example, *Ranunculus sceleratus* in order to achieve a more fundamental knowledge of the hormonal basis in the elongation reaction of amphibious species.

In contrast to auxin, a clear positive effect of gibberellin on submergence growth could be demonstrated in *R. palustris* (Table 8). The partial restoration of the paclobutrazol induced inhibition by  $\text{GA}_3$  could indicate that paclobutrazol also acts on other,  $\text{GA}_3$ -independent, growth. The remarkable differences between air-grown and submerged dose-response curves indicate that submergence leads to a higher responsiveness of the petioles for GA (Fig. 9). Increase of responsiveness of ethylene-treated shoots to gibberellin was mentioned by Kutschera and Kende (1988) for deepwater rice. Gibberellin might act *via* enhancement of tissue extensibility (c.f. increasing the cell wall yielding; Kutschera and Kende, 1988) by altering the direction of cellulose microfibrils (Sauter, Seagull and Kende, 1993).

To understand, in more detail, the role of ethylene, auxin and gibberellin in the adaptation reaction upon submergence, attention needs to be focused not only on the separate and combined actions of these hormones but also on processes at the molecular level. Differences in hormonal regulation might explain the different adventitious rooting capacity of the various *Rumex* species. Differences in the numbers of dormant root primordia between the species might well be important also. Extensive research on both anatomy and on regulatory phenomena of adventitious root formation is necessary to determine the value of each factor.

Apparently, low oxygen is one of the triggering factors for the adaptation reactions of submerged plants (see also Tonutti and Ramina, 1991; Crawford, 1992). Not only rooting may be stimulated at low oxygen conditions (Fig. 4), but also shoot elongation (Fig. 6). Preliminary findings strongly indicate that sub-ambient oxygen concentrations not only directly stimulate growth but also positively affect both ethylene production and tissue sensitivity, which was also demonstrated in roots of *Zea mays* L. by Brailsford *et al.*, 1993. Results presented in Table 7 clearly demonstrate that differences in submergence-induced growth between species are not only controlled by variation in endogenous ethylene levels, but also by different sensitivity towards this hormone.



The occurrence of phylogenetically related species on a gradient from lower to higher elevated sites in the river area offers an unique possibility to study the metabolic and physiological mechanisms underlying the ecological phenomena of plant distribution related to submergence and flooding. The integration of the two approaches taken in this study (the model or 'one genus—more habitats' approach and the screening or 'more species—one habitat' approach) are helping to elucidate adaptive processes at the level of individual organisms and also at the community level.

#### ACKNOWLEDGEMENTS

The authors thank Gerard Bögemann, Francis Huitink and Jos Michielse for their technical assistance in various phases of the work and Dr Gerard Barendse for fruitful discussions and suggestions. We thank Dr M. B. Jackson for critically commenting on the manuscript.

#### LITERATURE CITED

- Armstrong W. 1979. Aeration in higher plants. In: Woolhouse HW, ed. *Advances in botanical research*. London: Academic Press, 7: 225–332.
- Bienfait HF. 1989. Prevention of stress in iron metabolism of plants. *Acta Botanica Neerlandica* 38: 105–129.
- Blacqui re T. 1986. Nitrate reduction in the leaves and numbers of nitrifiers in the rhizosphere of *Plantago lanceolata* growing in two contrasting sites. *Plant and Soil* 91: 377–380.
- Blom CWPM. 1990. Responses to flooding in weeds from river areas. In: Kawano S, ed. *Biological approaches and evolutionary trends in plants*. London: Academic Press, 81–94.
- Blom CWPM, B gemann GM, Laan P, Van der Sman AJM, Van der Steeg HM, Voeselek LACJ. 1990. Adaptations to flooding in plants from river areas. *Aquatic Botany* 38: 29–47.
- Blom CWPM, Voeselek LACJ, Van der Sman AJM. 1993. Responses to total submergence in tolerant and intolerant riverside species. In: Jackson MB, Black CR, eds. *Interacting stresses on plants in a changing climate*. Berlin: Springer-Verlag, 243–266.
- Bollmark M, Eliasson L. 1990. Ethylene accelerates the breakdown of cytokinins and thereby stimulates rooting in Norway spruce hypocotyl cuttings. *Physiologia Plantarum* 80: 534–540.
- Both GJ, Gerards S, Laanbroek HJ. 1992. The occurrence of chemo-autotrophic nitrifiers in water-saturated grassland soils. *Microbial Ecology* 23: 15–26.
- Boutin C, Keddy PA. 1993. A functional classification of wetland plants. *Journal of Vegetation Science* 4: 591–600.
- Brailsford RW, Voeselek LACJ, Blom CWPM, Smith AR, Hall MA, Jackson MB. 1993. Enhanced ethylene production by primary roots of *Zea mays* L. in response to subambient partial pressures of oxygen. *Plant, Cell and Environment* 16: 1071–1080.
- Brown VK. 1992. Plant succession and life history strategy. *Tree* 7: 143–144.
- Cookson C, Osborne DJ. 1978. The stimulation of cell extension by ethylene and auxin in aquatic plants. *Planta* 144: 39–47.
- Crawford RMM. 1992. Oxygen availability as an ecological limit to plant distribution. *Advances in Ecological Research* 23: 93–185.
- Drew MC, Jackson MB, Giffard S. 1979. Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. *Planta* 147: 83–88.
- Engelaar WMHG, Bodelier PLE, Laanbroek HJ, Blom CWPM. 1991. Nitrification in the rhizosphere of flooding-resistant and a flooding-non-resistant *Rumex* species. *FEMS Microbiology Ecology* 86: 33–42.
- Engelaar WMHG, van Bruggen MW, van den Hoek WPM, Huyser MAH, Blom CWPM. 1993. Root porosities and radial oxygen losses of *Rumex* and *Plantago* species as influenced by soil-pore diameter and soil aeration. *The New Phytologist* 125: 565–574.
- Ernst WHO. 1990. Ecophysiology of plants in waterlogged and flooded environments. *Aquatic Botany* 38: 73–90.
- Gaynard TJ, Armstrong W. 1987. Some aspects of internal plant aeration in amphibious habitats. In: Crawford RMM, ed. *Plant life in aquatic and amphibious habitats*. Oxford: Blackwell Scientific Publications, 303–320.
- Grosse W, Meyer D. 1992. The effect of pressurized gas transport on nutrient uptake during hypoxia of alder roots. *Botanica Acta* 105: 223–226.
- Gurumurti K, Gupta BB, Kumar A. 1985. Hormonal regulation of root formation. In: Purohit SS, ed. *Hormonal regulation of plant growth and development*. Dordrecht: Martinus Nijhoff/Dr. W. Junk Publishers, 387–400.
- Hoffmann-Benning S, Kende H. 1992. On the role of abscisic acid and gibberellin in the regulation of growth in rice. *Plant Physiology* 99: 1156–1161.
- Horton RF, Samarakoon AB. 1982. Petiole growth in the celery-leaved crowfoot (*Ranunculus sceleratus* L.): effects of auxin-transport inhibitors. *Aquatic Botany* 13: 97–104.
- Jackson MB. 1982. Ethylene as a growth promoting hormone under flooded conditions. In: Wareing PF, ed. *Plant growth substances 1992*. London: Academic Press, 291–301.
- Jackson MB. 1985. Ethylene and the responses to soil waterlogging and submergence. *Annual Review of Plant Physiology* 36: 145–174.
- Jackson MB. 1990. Hormones and developmental change in plants subjected to submergence or soil waterlogging. *Aquatic Botany* 38: 49–72.
- Jackson MB, Fenning TM, Drew MC, Saker LR. 1985. Stimulation of ethylene production and gas-space (aerenchyma) formation in adventitious roots of *Zea mays* L. by small partial pressures of oxygen. *Planta* 165: 486–492.
- Jackson MB, Waters I, Setter T, Greenway H. 1987. Injury to rice plants caused by complete submergence; A contribution by ethylene (ethene). *Journal of Experimental Botany* 38: 1826–1838.
- Jensen CR, Luxmoore RJ, Van Gruny SD, Stolzy LH. 1969. Root air space measurements by a pycnometer method. *Agronomy Journal* 61: 474–475.
- Jongman RHG. 1992. Vegetation, river management and land use in the Dutch Rhine floodplains. *Regulated Rivers: Research and Management* 7: 279–289.
- Justin SHFW, Armstrong W. 1987. The anatomical characteristics of roots and plant response to soil flooding. *The New Phytologist* 106: 465–495.
- Kutschera U, Kende H. 1988. The biophysical basis of elongation growth in internodes of deepwater rice. *Plant Physiology* 88: 361–366.
- Laan P, Berrevoets MJ, Lythe S, Armstrong W, Blom CWPM. 1989a. Root morphology and aerenchyma formation as indicators of the flood-tolerance of *Rumex* species. *Journal of Ecology* 77: 693–703.
- Laan P, Blom CWPM. 1990. Growth and survival responses of *Rumex* species to flooded and submerged conditions: The importance of shoot elongation, under water photosynthesis and reserve carbohydrates. *Journal of Experimental Botany* 41: 775–783.
- Laan P, Clement JMAM, Blom CWPM. 1991b. Growth and development of *Rumex* roots as affected by hypoxic and anoxic conditions. *Plant and Soil* 136: 145–151.
- Laan P, Smolders A, Blom CWPM, Armstrong W. 1989b. The relative roles of internal aeration, radial oxygen losses, iron exclusion and nutrient balances in flood-tolerance of *Rumex*-species. *Acta Botanica Neerlandica* 38: 131–145.
- Laan P, Smolders A, Blom CWPM. 1991a. The relative importance of anaerobiosis and high iron levels in the flood tolerance of *Rumex* species. *Plant and Soil* 136: 153–161.
- Laan P, Tosserams M, Blom CWPM, Veen BW. 1990. Internal oxygen transport in *Rumex* species and its significance for respiration under hypoxic conditions. *Plant and Soil* 122: 39–46.
- Laanbroek HJ. 1990. Bacterial cycling of minerals that affect plant growth in waterlogged soils: a review. *Aquatic Botany* 38: 109–125.
- Malone M, Ridge I. 1983. Ethylene induced growth and proton excretion in the aquatic plant *Nymphaeodes peltata*. *Planta* 157: 71–73.



- Menges ES, Waller MW. 1983. Plant strategies in relation to elevation and light in floodplain herbs. *The American Naturalist* 122: 454–473.
- Musgrave A, Jackson MB, Ling E. 1972. *Callitriche* stem elongation is controlled by ethylene and gibberellin. *Nature New Biology* 238: 93–96.
- Musgrave A, Walters J. 1973. Ethylene-stimulated growth and auxin transport in *Ranunculus sceleratus* petioles. *The New Phytologist* 72: 783–789.
- Nordström A-C, Eliasson L. 1991. Levels of endogenous indole-3-acetic acid and indole-3-acetylserpic acid during adventitious root formation in pea cuttings. *Physiologia Plantarum* 82: 599–605.
- Osborne DJ. 1984. Ethylene and plants of aquatic and semi-aquatic environments. A review. *Plant Growth Regulation* 2: 167–185.
- Perik PJM, Voeselek LACJ, Blom CWPM, Sassen MMA. 1989. Morphological aspects of petiole elongation in submerged *Rumex* species. *Acta Botanica Neerlandica* 38: 349.
- Ponnamperuma FN. 1984. Effects of floodings on soils. In: Kozlowski TT, ed. *Flooding and plant growth*. New York: Academic Press, 10–45.
- Ridge I. 1987. Ethylene and growth control in amphibious plants. In: Crawford RMM, ed. *Plant life in aquatic and amphibious habitats*. Oxford: Blackwell Scientific Publications, 53–76.
- Robbins JA, Reid MS, Paul JL, Rost TL. 1985. The effect of ethylene on adventitious root formation in mung bean (*Vigna radiata*) cuttings. *Journal of Plant Growth Regulation* 4: 147–157.
- Samarakoon AB, Horton RF. 1983. Petiole growth in *Ranunculus sceleratus*: the role of growth regulators and the leaf blade. *Canadian Journal of Botany* 61: 3326–3331.
- Sauter M, Seagull RW, Kende H. 1993. Internodal elongation and orientation of cellulose microfibrils and microtubules in deepwater rice. *Planta* 190: 354–362.
- Schwegler T, Brändle R. 1991. Ethylenabhängige Wachstums- und Entwicklungsprozesse bei Stecklingen der Brunnenkresse (*Nasturtium officinale*). *Botanica Helvetica* 101: 135–140.
- Selby C, Kennedy SJ, Harvey BMR. 1992. Adventitious root formation in hypocotyl cuttings of *Picea sitchensis* (Bong.) Carr—the influence of plant growth regulators. *The New Phytologist* 120: 453–457.
- Smirnoff N, Crawford RMM. 1983. Variation in the structure and response to flooding of root aerenchyma in some wetland plants. *Annals of Botany* 51: 237–249.
- Smits AJM, Laan P, Thier RH, Van der Velde G. 1990. Root aerenchyma, oxygen leakage patterns and alcohol fermentation ability of the roots of some nymphaeid and isoetid macrophytes in relation to the sediment type of their habitat. *Aquatic Botany* 38: 3–17.
- Smulders MJM, Horton RF. 1991. Ethylene promotes growth and auxin promotes radial growth in *Ranunculus sceleratus* petioles. *Plant Physiology* 96: 806–811.
- Suge H. 1985. Ethylene and gibberellin: regulation of internodal elongation and nodal root development in floating rice. *Plant and Cell Physiology* 26: 607–614.
- Tonutti P, Ramina A. 1991. Oxygen concentration and ethylene production in roots and leaves of wheat: short term reaction in air after anoxic and hypoxic treatments. *Physiologia Plantarum* 81: 295–300.
- Van der Sman AJM. 1992. *Flooding resistance and life histories of short-lived floodplain herbs*. PhD Thesis. University of Nijmegen.
- Van der Sman AJM, Blom CWPM, Barendse GWM. 1993b. Flooding resistance and shoot elongation in relation to developmental stage and environmental conditions in *Rumex maritimus* L. and *Rumex palustris* Sm. *The New Phytologist* 125: 73–84.
- Van der Sman AJM, Joosten NN, Blom CWPM. 1993a. Flooding regimes and life-history characteristics of short-lived species in river forelands. *Journal of Ecology* 81: 121–130.
- Van der Sman AJM, Van Tongeren OFR, Blom CWPM. 1988. Growth and reproduction of *Rumex maritimus* and *Chenopodium rubrum* under different waterlogging regimes. *Acta Botanica Neerlandica* 37: 439–450.
- Van der Sman AJM, Voeselek LACJ, Blom CWPM, Harren FJM, Reuss J. 1991. The role of ethylene in shoot elongation with respect to survival and seed output of flooded *Rumex maritimus* L. plants. *Functional Ecology* 5: 304–313.
- Van der Valk AG. 1981. Succession in wetlands: a gleasonian approach. *Ecology* 62: 688–696.
- Van der Valk AG, Welling CH. 1988. The development of zonation in freshwater wetlands. In: During HJ, Werger MJA, Willems JH, eds. *Diversity and pattern in plant communities*. The Hague: SPB Academic Publishing, 145–158.
- Van Groenendael JM, Van Mansfeld MJM, Roozen AJM, Westhoff V. 1993. Vegetation succession in lakes in the coastal fringe of West Connemara, Ireland. *Aquatic Conservation: Marine and Freshwater Ecosystems* 3: 25–41.
- Voeselek LACJ. 1990. *Adaptation of Rumex in flooding gradients*. PhD thesis. University of Nijmegen.
- Voeselek LACJ, Blom CWPM. 1989a. Ethylene and flooding responses of *Rumex* species. In: Clijsters H et al., eds. *Biochemical and physiological aspects of ethylene production in lower and higher plants*. Dordrecht: Kluwer Academic Publishers, 245–253.
- Voeselek LACJ, Blom CWPM. 1989b. Growth responses of *Rumex* species in relation to submergence and ethylene. *Plant, Cell and Environment* 12: 433–439.
- Voeselek LACJ, Harren FJM, Bögemann GM, Blom CWPM, Reuss J. 1990. Ethylene production and petiole growth in *Rumex* plants induced by soil waterlogging. *Plant Physiology* 94: 1071–1077.
- Voeselek LACJ, Van der Sman AJM, Harren FJM, Blom CWPM. 1992. An amalgamation between hormone physiology and plant ecology: A review on ethylene and flooding resistance. *Journal of Plant Growth Regulation* 11: 171–188.
- Voeselek LACJ, Van Oorschot FGMM, Smits AJM, Blom CWPM. 1993a. The role of flooding resistance in the establishment of *Rumex* seedlings in river flood plains. *Functional Ecology* 7: 105–114.
- Voeselek LACJ, Banga M, Thier RH, Mudde CM, Harren FJM, Barendse GWM, Blom CWPM. 1993b. Submergence-induced ethylene synthesis, entrapment, and growth in two plant species with contrasting flooding resistances. *Plant Physiology* 103: 783–791.
- Walters J, Osborne DJ. 1979. Ethylene and auxin-induced cell growth in relation to auxin transport and metabolism and ethylene production in the semi-aquatic plant *Regnellidium diphyllum*. *Planta* 146: 309–317.
- Yamamoto F, Kozlowski TT, Wolters KE. 1987. Effect of flooding on growth, stem anatomy, and ethylene production of *Pinus halepensis* seedlings. *Canadian Journal of Forest Research* 17: 69–79.
- Yamamoto F, Kozlowski TT. 1987a. Effects of flooding, tilting of stem and ethrel application on growth, stem anatomy, and ethylene production of *Acer platanoides* seedlings. *Scandinavian Journal of Forest Research* 2: 141–156.
- Yamamoto F, Kozlowski TT. 1987b. Effects of flooding of soil on growth, stem anatomy, and ethylene production of *Thuja orientalis* seedlings. *IAWA Bulletin* 8: 21–29.
- Yamamoto F, Kozlowski TT. 1987c. Regulation by auxin and ethylene of responses of *Acer negundo* seedlings to flooding of soil. *Environmental and Experimental Botany* 27: 329–340.